# Bacterial contamination and nutritional adequacy of enteral tube feedings in Iran

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Summary. Background: In spite of recent advances in nutritional support, researches are often unclear and, in many cases, conflicting in regard to the most appropriate formulas. This study aimed to evaluate nutritional adequacy and bacterial contaminations of enteral feedings (EFs) that are used in the intensive care units (ICUs) of hospitals in Tabriz, Iran. Methods: This experimental study was carried out on 54 EFs samples; 36 blenderized tube feedings (BTFs) and 18 commercial powder feedings (CPFs) of patients in the ICUs. Energy and macronutrients contents of formulas were measured and compared with estimated needs of patients. Thirty-six BTFs samples (18 after preparation and 18 after 18 hour keeping in refrigerator) and 18 CPFs samples immediately after preparation were tested for the presence of total coliforms, Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Listeria monocytogenes (L. monocytogenes), and Salmonella. Results: The energy density (ED) of BTFs (0.74±0.02 kcal/mL) were higher than CPFs (0.59±0.02 kcal/mL) and both lower than predicted values (p<0.001). The energy and macronutrients content were significantly different (p<0.001) between BTFs and CPFs. Total coliforms of BTFs were less than 2 MPN/gr in both times, but 6 (33%) of CPFs samples were 6.41±2.43 MPN/gr. E. coli contamination were detected only in CPFs and S. aureus, Salmonella, and L. monocytogenes in both EFs were not detected. Conclusion: Given that low energy and macronutrient contents of both types of EFs, and bacterial contamination of CPFs, it is necessary to pay attention to the quality, safety, and appropriate type of formulas.

Key words: enteral nutrition, nutritional support, enteral formula

# Introduction

About 30%-60% of patients admitted to hospitals are not in good conditions and they have degrees of malnutrition (1). This is about 48% in our country (2). Proper nutritional intervention is the first step to prevent malnutrition. Nutritional support strategies include adding supplements to the diets, enteral feeding (EF) - commercial prepared formulas or hospital prepared feeds- and partial or total parenteral nutrition (3, 4).

EF was started in the early 20th century and has become one of the most common and preferred meth-

ods of nutritional support in patients with a functional gastrointestinal tract, that cannot satisfy their nutritional requirements. Despite worldwide access to commercial formulas, some still prefer to use blenderized tube feedings (BTFs). BTFs contain natural foods such as milk, egg, meat, soft fruit, oils, and vegetables. Economic reasons and/or lack of easy and inexpensive access to commercial feedings, cultural issues and flexibility in the preparation of BTFs can be the most important reasons justify the use of these formulas (4-10).

Whole food/blenderized formula only considered for use in medically stable patients with no signs of in-

fection; best suited for patients with safe food practices and tube maintenance techniques; should be provided as bolus feeds to maintain safe food practices, and registered dietitians should be involved in development of feeding composition to ensure adequate nutrient delivery (11). The most important issues that should be considered are microbial contamination and nutritional quality of BTFs (12). Contamination of Efs has been studied in many countries (9, 12-16) and can be occurred in all stages of production, preparation, storage, and administration process to the patients. Bacterial contamination prevalence of BTFs were reported as much as 30%-57% of samples (17). Contaminated feedings increase the risk of nosocomial infections such as diarrhea, pneumonia and septicemia (18). In the Philippine, contamination of 75-96% EFs was >10<sup>4</sup> colony-forming units (CFU)/gr (using standard plate count) (19). This was in Saudi Arabia, over than 10<sup>4</sup> CFU/gr (9).

In addition to bacterial contamination EFs must have nutritional balance to provide the appropriate energy and nutrients. Commercial feedings have specified composition and osmolality but handmade feedings vary in composition depending on the food stuffs and preparation procedures. Many studies have shown that levels of macronutrients and micronutrients are unpredictable and inconsistent (9, 20, 21).

Because of increasing need to personalized services of Efs and regarding nutritional and health concerns; this study designed to evaluate and compare nutritional adequacy and microbial contamination of BTFs and commercial powder feedings (CPFs) in intensive care units (ICUs) of Tabriz hospitals.

## Methods

This experimental cross-sectional study was carried out on 54 EFs samples; 34 BTFs and 18 CPFs of patients in the ICUs of Imam Reza and Shohada hospitals during different days within two months in Tabriz, the capital city of East Azerbaijan Province in North West of Iran. Patients with an age range 20-70 years visited by nutritionist to determine the nutritional needs, then who have EFs by only BTFs or CPFs were selected. Diets designed by nutritionist for each patient based on their nutritional assessment. Energy requirements of patients were calculated by the Mifflin equation (22). BTFs used in this study, were prepared in the specific clean room under nutritionist supervision. All BTFs foodstuffs (table1) weighed, blenderized, strained, and immediately transferred to the wards refrigerators for gradual administration according to physician's orders up to 24 hrs.

In this study ready to use enteral powder (Enterameal) that prepared according to physician order and instructions on the can by nurses were used in ICUs. Ingredients of Enterameal powder was protein with high biological value, carbohydrates (maltodextrin), Inulin, Gluten-free, sun flower, canola and coconut oil that can provide much of daily needs of patients. The two forms of packaging available, Cans (400 g) and Sachet (26.5 g). In our study the type of used was cans (400 g) which kept beside patients beds.

In the period of 2 months a total number of 36 BTFs samples (18 samples immediately and 18 sample 18 h after preparation); and 18 samples of CPFs immediately after preparation at the wards just before administration were collected from the ICUs. All samples (250 mL of feeds) were collected in plastic closed container for energy and macronutrients measurement and 100 mL of both EFs were collected in sterile sealed glass containers for microbial analysis, and then transported to the microbiology laboratory in Tabriz University of medical sciences in an icebox for microbiological analysis.

Tabella 1. Food composition of blenderized formula

Food groups	Foodstuffs		
Dairy	Lactose free powder milk, and low		
	fat yogurt		
Protein	Cheese, cooked chicken, egg, and lentil		
Starch	Boiled potatoes, rice flour, and		
	wholegrain biscuit		
Vegetable	Cucumber, cooked carrot, and		
	cooked tomato		
Fruit	Peeled apple, tangerine, orange,		
	and banana		
Fat	Olive oil and corn oil		

Macronutrients measured by Kjeldahl for protein, digestion method for carbohydrate and Soxhlet for lipids. Energy and macronutrients contents of samples reported in 100 mL. The results compared with researcher predicted values of patients' needs.

Coliform, and Staphylococcus aureus (*S. aureus*) count; and presence of *Salmonella* spp; *Listeria* spp; and *Escherichia coli* (*E. coli*) tests for all samples were conducted. Samples were analyzed to determine the number of Coliforms and *S. aureus* by using MPN/gr (most probable number) and pure plate technique respectively.

For S. aureus quantification, 0.1% sterile buffered peptone water was prepared. From each dilution a 1 mL aliquot was added to Baird-Parker Agar (BPA, Merck), then incubated at 37°C for 24-48 h. Then specific biochemical tests were performed on colonies obtained on BPA. For Coliform count used 5 tube fermentation or MPN method. After preparation of 0.1% phosphate buffer, from this dilution 10 mL was added to lactose broth which containing Durham tubes for presumptive phase and incubated at 37°C for 24-48 hrs. For confirmed phase use brilliant green bile lactose broth or BG broth with Durham tubes and incubated at 37°C for 24-48 hrs. Growth (turbidity) and gas production confirmed the presence of total coliform in the tubes. For completed phase used positive tubes of validation stage on EMB Agar medium and incubated at 37°C for 24 h. If coliform colonies with or without metallic luster to dark red or pink are seen on the medium it means to ensure the presence of total coliform.

For the presence of Listeria spp. and particularly for Listeria monocytogenes using selective enrichment and isolation protocol (12). Twenty-five grams of samples were taken and homogenized for 2 minutes in 225 mL of UVM Listeria enrichment broth (UVM I) (Difco, America) and incubated at 30°C for 24 hrs. One mL of primary enrichments was transferred to 9 mL of UVM II (Fraser broth) (Amyl Media, Australia) incubated at 35°C for 48 hrs. Then Secondary enrichments were streaked on Oxford Agar (Merck) and Palcam Agar (Merck) and incubated at 37°C for 48 hrs. Then plates examined for typical Listeria colonies (black colonies with black sunken) were sub cultured on Trypton Soy Agar supplemented with 0.6% of yeast extract (TSAYE) and incubated at 37°C for 24 hrs. Then standard biochemical tests such as Gram's stain, catalase test, motility at 25°C and 37°C, acid production from glucose, manitol, rhamnose, xylose, E- methyl-D-manoside, and nitrate reduction, hydrolysis of esculin and MR/VP test were done. For further confirmations of *Listeria* spp. and other biochemical reactions,  $\beta$ -haemolytic activity, and CAMP test were performed according to the Bergey's Manual of Systematic Bacteriology. Samples were also examined for the presence of *Salmonella* spp.

Organization for the isolation of Salmonella first a 25 g portion of each sample in a sterile stomacher bag containing 225 mL sterile Buffered Peptone Water (BPW) was weighed and shaken for 2 minutes. BPW was used for pre-enrichment at 37°C for 18-24 h. Then 1 mL of the pre-enriched sample was inoculated into 10 mL of Modified Rappaport- Vassiliadis (RV, Merck) broth and 9 mL of Selenite Cystine (SC, Merck) broth and was incubated at 42°C and 37°C respectively for 24 h. Xylose Lysine Deoxycholate (XLD, Merck) medium was used as selective isolation media and incubated at 37°C for 24 hrs. From each plate at least three characteristic colonies were picked and purified by streaking on Tryptone Soy Agar (TSA, Merck). Cultures were further subjected to analysis for Gram's stain, motility, ONPG, urease, lysine decarboxylase and reaction on Triple Sugar Iron Agar. Results were expressed in presence or absence of Salmonella or Listeria.

For the *Escherichia coli* presence the contents of the positive test tubes from the coliform test were spread over the surface of Levine agar plates. After incubation at 37°C for 24 h colonies typical of *E. coli* were identified. Results are expressed as presence or absence of *E. coli*.

#### Statistical analysis

The influential variables were adjusted between two intervention groups (BTF and CPF) at the beginning of the study. For each person, the data was calculated in two measurement methods provided and estimated. For quantitative data, normality was evaluated by Q-Q test and then Mauchly's W test was checked for identity covariance matrix, finally repeated measure with control covariates test was used by Minitab Software version 17. The results include five P-values for comparing groups and sub groups in multi and uni variates. The first was P-value<sub>Group</sub> for comparing variations in two intervention groups (BTFs and CPFs), the second was, P-value<sub>Subgroups</sub> for comparing between two measurement methods (provided and estimated) in each group (BTFs and CPFs) synchronously. The third till fifth P-values used for controlling gender, age and BMI as confounding variables. Paired T-test was used for comparing between subgroups (provided and estimated) in each group (BTFs and CPFs) respectively. The level of significance was set at 0.05 and all results were expressed as Mean±SEM (standard error of mean).

# Results

Demographic information of patients in ICUs is shown in table 2. According to that, at the beginning of the study, there wasn't any significant difference in age, gender and BMI between two groups of patients in ICUs. As shown in Table 3, the energy density (ED) of BTFs was higher than CPFs, but ED of both groups was lower than predicted value (Table 3). The ED/mL in both BTF and CPF groups was significantly different between provided and predicted values (P<0.001). There was statistically significant difference (P<0.001) between provided and estimated needs of energy (kcal/day), in CPF group but not in BTF group (P=0.799). Also provided energy in CPF group was significantly lower than BTF group (P<0.001). After adjusting the confounding factors such as gender and age (BMI was not considered as a confounder), the significant difference of energy contents (kcal/day) between two groups was confirmed by repeated measure test (P<0.001).

Lawley-Hotteling test showed (Table 3) that in macronutrients category, none of the age, sex, and BMI variables did not act as confounding factors and at least one of the variables (carbohydrate, protein and fat) was

Tabella 2. Demographic characteristics of patients with ent	eral
nutrition	

Group	BTF	CPF	Pv
Gender: M/F	50%/50%	55.60%/46.40%	0.738
Age (year)	56.94±3.35	62.44±3.00	0.229
BMI	24.76±0.53	24.26±1.03	0.672
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BTF: Blenderized tube feeding; CPF: Commercial Powder Formula; BMI: Body mass index

Tabella 3. Mean ± SE of nutritional facts of enteral feedings in ICU patients

Group		BTF (n=18)			CPF (n=18)			P	7		
Sub Groups	Provided	Estimated	* <b>P</b> <sub>v</sub>	Provided	Estimated	* <b>P</b> <sub>v</sub>	Repeated	Measure	Confo	unding	Factors
							Group	Sub Groups	Sex	Age	BMI
Energy (kcal/day)	1499.81±30.33	1509.12±44.67	0.799	1100±31.44	1527.29±41.87	0.000	0.001	0.000	0.045	0.039	0.389
ED (kcal/mL)	0.76±0.02	1±0	0.000	0.59±0.02	1±0	0.000	0.000	0.000	0.415	0.570	0.639
Cho (g/100 mL)	10.87±0.22	12.73±0.12	0.000	8.04±0.14	12.58±0.04	0.000	0.000	0.000	0.743	0.213	0.503
Pro (g/100 mL)	3.81±0.11	5.11±0.03	0.000	2.94±0.13	4.92±0.04	0.000	0.000	0.000	0.828	0.162	0.132
Fat (g/100 mL)	1.98±0.02	3.22±0.04	0.000	2.25±0.02	3.29±0.02	0.000	0.000	0.000	0.564	0.664	0.222
Lawley-Hot	elling Test						0.000	0.000	0.874	0.273	0.337

Microbial count	BTH	CPF (n=18)	
	Preparation time (n=18)	18 h after preparation (n=18)	Preparation time
Coliform	<2 MPN/g	<2 MPN/g	6.41±2.43 MPN/g
S.aureus	<10 <sup>1</sup> cfu/g	<10 <sup>1</sup> cfu/g	<10 <sup>1</sup> cfu/g
Salmonella	absence	absence	absence
L.monocytogenes	absence	absence	absence
E.coli	absence	absence	existence

Tabella 4. Bacterial contamination of blenderized and commercial formulas

BTF: Blenderized tube feeding; CPF: Commercial powder feeding; MPN: Most probable number; S.aureus: Staphylococcus aureus; L. monocytogenes: Listeria monocytogenes; CFU/g: colony forming count per gram

significantly different (P<0.001) with others. Macronutrients contents (g/100 mL/day) between groups (BTFS and CPFs) and subgroups (provided and estimated needs) were significantly different (P<0.001).

Bacterial contamination (*S. aureus*, coliform) of BTFs at the preparation time and 18 hrs after preparation were <10<sup>1</sup> cfu/ and <2 MPN/gr, in fact they didn't have contamination but 6 samples of 18 (33%) CPFs which prepared in the wards have coliform contamination 6.41±2.43 MPN/gr and *E. coli* were detected. No Salmonella spp. and L. monocytogenes were detected from any samples of CPFs at the time of preparation and in BTFs were not detected any *Salmonella* spp., *L. monocytogenes* and *E. coli* at the preparation and 18 hrs after storage in the wards.

### Discussion

Proper nutritional support is one of the earlier step in preventing malnutrition in ICU patients (3). Given to the prevalence of hypermetabolism and malnutrition in these patients, providing adequate energy and protein are very important to achieve best results (4, 10, 23). Although commercial, ready-to-use formulas have been available for over 20 years, but because of many reasons BTFs are used in many institutions to fed patients because of perceived economic advantages or cultural preferences, perceived health benefits, intolerance to CPFs, food allergies, improved bowel function, psychosocial reasons, or personal preference (desire for "real" food, organic, vegetarian, etc.). But BTFs usually have lower ED and need to higher volume of the solution. Viscosity, osmolality and microbial contamination of BTFs are the other problems of them (8-10).

The results of this study highlight some of the advantages and disadvantages of BTFs and CPFs. In contrast to CPFs, considering liberal selecting the type and amount of food stuffs, BTFs was flexible in meeting patient individual nutritional needs.

Unexpectedly we found that ED and macronutrients contents of CPFs were less than predicted and BTFs values. BTFs and standard CPFs usually design to contain approximately 1 kcal/mL. Considering that the sampling time, it seems that the reason for conflicting results between ED and total daily energy (provided and estimated) was non-compliance with the instructions and dilution of formulas to facilitate administration.

Previous studies showed similar results regarding ED and macronutrient distribution (9, 20, 21, 23-27). Jalal K. Mokhalalati reported that BTFs did not provide the predicted nutrient content. They stated that there are several likely sources for the variability, including human error and inconsistencies in measuring, as well as loss of nutrients in cooking and processing foods, which, again, will vary depending on the personnel preparing the food (9). Inadequate caloric and protein intake was common on Isidro and Lima study (27). In one study, conducted by Sullivan et al in Philippine the results showed that BTFs render unpredictable levels of energy and macronutrients and appear likely to deliver less than the desired amounts of nutrients (21). Salehifar et al reported that mean of energy intake was 0.39±0.164 kcal/mL and protein intake was very low (24). Results of this study were in line with Safarian et al in terms of meeting ED and the most of macronutrients estimated by BTFs (28).

Other potential complication of enteral feedings is microbial contamination of the solution. In the event of failure to comply with health standards in the preparation and maintenance of the solution, they are suitable for the growth of various microorganisms (16).

Contaminations of EFs have associated with nosocomial infections, diarrhea, bacteremia, and pneumonia. Recent guidelines of Food and Drug Administration (FDA) regarding microbial quality of medical foods, including tube feeding formulas, stated that action must be taken if any such products contain more than  $10^4$  cfu/g or if three or more samples exceeded  $10^3$ CFU/g. Also they limit the acceptable level of coliforms to 3 organisms/g (12). According to this definition, the results of present study showed that BTFs were safe and had no contamination at the time of preparation and 18 hrs after keeping in refrigerator, but CPFs which prepared in the hospital wards had coliform (6.41±2.43 MPN/gr) and E. coli contamination. According to above standards the existence of *E*. coli and coliforms in 33.3% of CPFs were unacceptably contaminated.

In the field of microbial contamination of EFs many studies have been conducted (9, 12, 14, 15, 19, 20, 26, 29, 30). In most of these studies microbial contamination was above of standard values. The results of the microbiological analysis of enteral feeding solution was prepared by hand in the Philippines showed that 75-96% of microbial counts of samples were more than 101 CFU/gr (19). A study in Saudi Arabia was indicated that nearly all BTFs samples had aerobic microbial count >104 CFU/g and all of them had coliform contamination but CPFs had no coliform and aerobic bacterial contamination (<10<sup>1</sup> CFU/g) (9). According to Isfahan studies Efs were highly contaminated and not safe to use (12). But in one of these studies; such as our results; contamination of ready-to-use formulas was significantly more than that for handmade samples (31).

Studies mentioned some reasons about low energy and different macronutrient distribution and microbial contamination. The bag-type container and the infusion tube, both of which are contamination factors in later stages so they should be used only once or only in one patient for a maximum of 24 hrs (29). Handling of any part of the feeding administration apparatus during assembly or use may result in contamination, which can be reduced by meticulous hand washing; putative sources being hospital staffs, ventilators, suction apparatus, wash bowls and the patient him/herself (endogenous flora of the gut, upper respiratory tract, and skin) (19).

Inadequate levels of staffing in the hospital kitchen, poor training, low wages and lack of support from managers is one of the reasons of contamination of hospital feedings (30).

The lake of BTFs contamination in our study either the preparation or after 18 hrs keeping in refrigerator, was because of adequate levels of staffing in the preparation room, good training, the use of disposable gloves, proprietary clean room, meet the hygiene standards, use of boiled water in preparation of solutions, supervising dietitian, special and trained staffs for preparation, immediate transferring and keeping of BTFs into the ward's refrigerators in closed containers. Coliform contamination of CPFs may be because of secondary pollution (hands of nurses that prepare the feedings) or because of polluted devices that kept in the ICU wards to mixing powder or water which used to prepare solutions, because these types of bacteria is associated with the fecal-hand route of transmission and they are unstable to heat so should not exist in the solution are heated.

To reduce contamination of CPFs it's better to prepare them in specific clean room by trained staffs and use the type of the sachet of Enterameal instead of 400gr cans. Better attention to hygiene, both personal and food hygiene, and adherence to HACCP principles would have prevented many outbreaks. Implementation of the HACCP, improved significantly the microbial quality of the feeds, with counts of 10<sup>1</sup>cfu/mL that before implementation of the HACCP was >10<sup>4</sup> CFU/mL (13). Malnutrition (under/over nutrition) could impacts patient's health on ICUs, so providing accurate and appropriate nutritional requirements of these patients must be taken serious proceedings. Lack of attention to the nutritional needs of ICU patients lead to exacerbations of disease, mechanical ventilation dependence, and increasing costs, and length of stay in hospitalization (8).

Increase supervision of a nutritionist, more accuracy in weighing of foodstuffs, and preparing formulas by trained staffs is some ways to improve BTFs macronutrients and ED. It seems use the type of sachet Enterameal is better than cans. Ready to use CPFs solutions instead of powders that don't need to add water and mix seems better because reduce errors in preparation.

However, not only does the superiority of specialized over standard enteral formulas remain insufficiently substantiated, but there are no firm data supporting the clinical benefits of commercial diets over blenderized food (32, 33).

Considering the importance of nutritional support for patients in ICU, teaching nutrition and coordination between members of the medical staff including physicians, clinical pharmacists, nurses and dietitian to assess the nutritional status of patients and the provision of energy and protein needs, given the specific circumstances each patient is necessary and can help to improve the care of these patients. Main limitation of this study was lack of routine use of CPFs in ICUs because of economic issue.

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